PHYTOCHEMICAL VARIATIONS OF BLACK POPLAR (*POPULUS NIGRA*), METABOLIC ANSWERS AND POPULATIONAL STRUCTURE OF THE APHID *CHAITOPHORUS LEUCOMELAS* (KOCH, 1854) (HOMOPTERA: APHIDIDAE)

DJAZOULI ZAHR-EDDINE[1], ZAHRAOUI ABDERRAHMANE AMINE[2] & PETIT DANIEL[3]

[1,2]Université De Blida 1, Faculté Des Sciences De La Nature Et De La Vie, Département

Des Biotechnologies, Route De Soumaa Blida, Algérie

[3]UMR 1061, INRA, Université de Limoges, 128, av. A. Thomas, Limoges Cedex France, Limoges, France

ABSTRACT

The black poplar *Populus nigra* is usually composed of only one cultivar, and is prone to attacks from bio-aggressors, of which the most damaging is the aphid *Chaitophorus leucomelas*. In order to determine the dynamics and the temporal succession of the various phenotypical forms, two areas of Algeria were selected, Zéralda (coastal area) and Médéa (mountainous area). The fitness and the populational structuring of the various generations were assessed by the quantification of female fecundity and the energy reserves in relation to the seasonal phytochemical quality of the host plant. The results revealed a temporal shift in the succession of the various phenotypes between the coastal area and the mountainous area. They also indicate a variability in the lipidic and carbohydrates content in the various phenotypes in relation to the seasonal changes of the phytochemical quality of *P. nigra*. Lastly, the results show a clear correlation between total sugars present in plant leaves, the modulation of the energy reserves and fecundity of aphid phenotypes. The populations undergoing more stress from dryness spend more energy to maintain homeostasis and as a consequence show lower fecundity.

KEYWORDS: Black Poplar, *Chaitophorus leucomelas*, Phenotype Succession, Energetic Molecules, Condensed Tannins, Total Sugars, Fecundity

INTRODUCTION

The plants, due to their inability to move, are subjected to numerous biotic or abiotic stresses in their biotope. The various causes of aggression from the environment are likely to disturb the development of trees and to affect their agronomic potentialities in term of production. The biotic aggressions in agro-system can occur through interactions with insects, nematodes, mushrooms, bacteria, virus, and adventitious herbs (Deshayes, 1991). According to Schultz (2002), the insects consume around 10% of plant production in the natural systems and are responsible for 15% of harvest losses in the world.

The poplar is a tree model which allows to tackle several questions dealing with land (Aronson & Le Floc'h, 1996) and alluvial space management (Chevallier, 2000), sawlog production (Breton, 2000), and use in phytoremediation (Di Lonardo *et al*., 2011). The poplar stands are usually composed of only one cultivar, and as a result are very vulnerable, much more than the traditional forest species whose settlements comprise genetically polymorphic populations (Bourdu, 1986). In consequence, the parasitic attacks can thus be numerous, repeated and very

harmful, among which *Phyllocnistis unipunctella* and *Chaitophorus leucomelas* (Delplanque, 1998; Djazouli *et al.*, 2009) can be pointed out. *Chaitophorus leucomelas* represents a real danger to poplar plantations but very few studies were done about its adaptation to different geographical altitudes in terms of populational structuring and life cycle.

The study of the different phenotypes of *Chaitophorus leucomelas* is little documented. The only works devoted to the biological cycle of *C. leucomelas* have been limited to a brief description of the various aphid generations on *Populus nigra* in the American continent (Ortego *et al.*, 1999 ; Giganti *et al.*, 2004). Moreover, Petrović-Obradovic *et al.* (2010) have described certain aphid phenotypes and their corresponding damages on the host plant. To our knowledge, no information is available for this aphid species in the Mediterranean basin. The question of describing the biological performances of *C. leucomelas* requires fine studies. Djazouli *et al.* (2011) highlighted the modulation of energy reserves of fundatrigeniae of *C. leucomelas* under the effect of the thermal variations. It remains that very few studies were made on both populational structuring and metabolic answer according to different altitudes.

We compared aphid populations taken at two sites of North Algeria. The first one, Médéa is considered as reference station in the sense of more favorable environmental conditions (altitude around 1000 m and annual rainfall= 852.23 mm). The second one is Zéralda (6 m of altitude and P=620.27 mm, with moderate seasonal variations of temperature). The present study was initiated in order to compare the succession of the different phenotypes in both stations, in terms of lag and abundance. This last feature was assessed by the only measure of fecundity. Among the underlying factors explaining these eventual differences, we considered some biochemical parameters of the host plant.   We also were interested in the energetic metabolism of aphids themselves because   Diallo & Djazouli (2010) showed a link between the metabolic answers of *C. leucomelas* to the qualitative phytochemical variations of *Populus sp*. in the sub-littoral and littoral areas of Algeria. In addition, several works correlated the nutritional quality of the host plant and the reproductive success of the individuals (Delisle & Bouchard, 1995; Delisle & Hardy, 1997; Awmack & Leather, 2002). Scriber & Slansky (1981) have also described the abundance of the different generations in relation to the nutritional value of the foliage. Our strategy was to predict the metabolic answers of aphids in the low altitude station from the data obtained in the higher altitude one, and to deduce their fecundity. If the predicted values are with the range of observed values,   it will be concluded an absence of stress, without specific response. In contrast, if discrepancies between observed and predicted values are recorded, it will imply a disturbance of metabolism limiting the fitness of certain phenotypes.

MATERIALS AND METHODS

Presentation of Study Sites and Bioclimatic Parameters

Algeria is a country subjected to the combined influence of the sea, relief and altitude. The climate is of Mediterranean type, characterized by one long period of summer dryness varying from 3 to 4 months on the littoral, from 5 to 6 months on the High Plains, and more than 6 months in the Saharian Atlas (Nedjraoui, 2003). Our study was carried out in two sites in North Algeria, precisely in the littoral area of Zéralda (36°41N-2°50E) and in the Tellian Atlas at Médéa (45°55'6"N - 13°25'15"E). Zéralda is a commune of the Algiers Wilaya, located at 29 km west of its capital. This small town has an agricultural vocation, and is located on a vast plain of the Algiers Sahel with a light declivity toward sand beaches, delimited in the West by Douaouda and Koléa, in the North-East by Staoueli and Souidania, and in South-East by Mahelma. Médéa is located at 80 km in the South-West of Algiers, in the middle of Tellian Atlas, on the high plateaus which close the valley of Mitidja. The area is characterized by an elevation around 1000 m A.S.L. and a strong relief

enclosing some fertile plains, but with a weak extension, ending in the borders of the high steppe plains, in a series of weakly corrugated hills. It is limited to the North-East by Hamdania, to the North-West by Tamezguida, to the East by Draa Esmar, to the West by Ouzera, and the South by Damiet (Grimes *et al.,* 2005). The area of Zéralda (Figure 1) presents a five-month long dry period between May and October, with the maximum mean temperature in August (M=31.72°C). The monthly rainfall is very variable throughout the year and ranges between 199.66 mm in December and 0.88 mm in July, and the annual cumulating value is P=620.27 mm. As for the area of Médéa (figure 1b), the dry period is spread over three months, whereas the humid period is longer than that at Zéralda, from September to June. The mean minimal temperature is recorded in February (m=4.12°C), *i.e.* lower than that of Zéralda of approximately 4-5°C. Cumulated annual rainfall is P=852.23 mm. As a synthetic view, the Q_2 index of Emberger-Sauvage (1963) classifies the area of Médéa in the sub-humid stage with mild winter, and the area of Zéralda in the sub-humid stage with warm winter (Q_2=3.43 P/M-m).
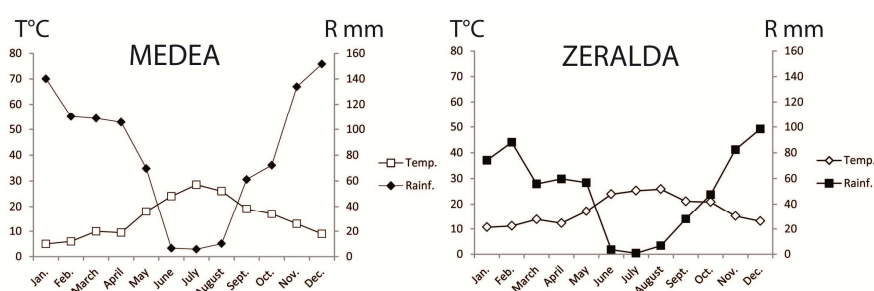

Figure 1: Ombrothermic Diagrams (1995-2009) at Zéralda (a) and Médéa (b)

Biological Material

Our experiments were carried out in 2009-2010 on leaves of *Populus nigra* collected in artificial poplar stands at Zéralda and Médéa. The poplar settlement of Zéralda is 18 years old, and extends on an area of approximately 3 ha, at an altitude of 6 m A.S.L., distant from the coast by around 500 m. The settlement of Médéa is 15 years old, distant of 80 km from the sea, and spreads out over 4 ha, at 1036 m A.S.L.

For our entomological study, we took the individuals of the various phenotypes of *Chaitophorus leucomelas* (Aphididae, Homoptera) in the areas of Médéa and Zéralda during 12 months. The samplings concerned sexuparae (Figure 2b) during the autumnal period, sexual oviparae (Figure 2d) during winter period, the fundatrix (Figure 2a) and fondatrigeniae (Figure 2e) during the spring period, and the virginoparae (Figure 2c) during the summer period.
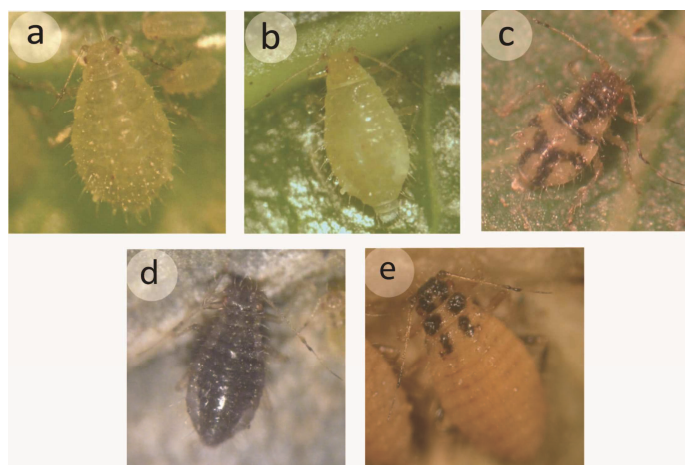
Figure 2: Variations of *Chaitophorus Leucomelas* Females (G x20)
a: Fundatrix, b: Sexuparae, c: Virginoparae, d: Sexual Oviparae, e: Fundatrigeniae

Sampling was carried out according to the transect method of Frontier (1983). From each of 16 selected trees, we took two leaves in each cardinal direction, with an interval of 10-15 days during the period of investigation, spread over 12 months. The insect material collected during each day of sampling was checked, weighed and put in 1.5 ml already weighed Eppendorf tubes, and then kept at -20°C for further analyses. The collected plant material was preserved at -20 °C for further phytochemical assays.

Extraction and Measurement of Energetic Reserves

The lipidic and carbohydrates biomarkers were quantified in the sampled aphids. The extraction and assay of the lipidic reserves were carried out according to the method of Van Brummelen & Suijfzand (1993). Briefly, a monophasic mixture 1:2:0.8 (chloroform: methanol: bi-distillated water) is versed in the tubes containing the aphids and used as solution of extraction.

For the extraction and the quantification of the carbohydrates reserves, we followed the method of Win Decoen (2000). According to this author, the extraction and essay of sugars in insects are generally done from the hemolymph. In our assays, because of the small size of aphids, sugars were extracted from the insects in their entirety.

Extraction Method and Assay of the Main Phytochemical Products

Total soluble sugars were assayed using the method of Dubois *et al.* (1956). The plant leaves are put in contact with ethanol at 80% during 48 hours at room temperature, and then dried in an oven at 80°C. When alcohol is evaporated, 20 ml of distilled water is added to the residue. A fraction of 2 ml of the obtained solution is added with phenol at 5%, sulphuric acid at 96%, then homogenized with vortex, after 10 min; This fraction is then placed in a water-bath at a temperature of 30°C during 20 min; 10 min later, the optical density is read at 485 nm.

The method used for extraction and assay of proline followed that of Troll & Lindsley (1955), modified by Dreir & Goring (1974), and starts from fresh plant leaves mixed with methanol, heated at 85°C during 60 min. After cooling, the extract is added to acetic acid, ninhydrin and a mixture of distilled water, acetic acid and orthophosphoric acid (0.4: 1: 0.26); then it is boiled during 30 min, until the color turns to red. After cooling, the addition of toluene leads to the separation of the solution in two phases: the higher phase containing the proline is recovered, to which Na_2SO_4 is added and the optical density is read at 528 nm.

The extraction and assay of condensed tannins followed the method of Price *et al.* (1978). A double extraction is carried out by a mixture of acetone and distilled water (7: 3) and of the plant powder of the leaves finely crushed. The obtained filtrate is evaporated under low pressure until desiccation. The dry residues are moistened by 5 ml of hot methanol. A mixture of methanolic tannins and vanillin-HCl solution (1:5) is heated with water-bath during 20 min at 30°C, and the absorptance is read at 535 nm.

According to Ben Naceur (1994), the water relative content is deduced from the following formula: WRC = (FW - DW) 100 / (SW – DW), with FW: Fresh weight, DW: Dry weight, SW: saturation weight. The fresh weight is determined by weighing of the leaves immediately after sampling. The saturation weight is obtained by placing the leave in distilled water during 24 hours. The dry weight is obtained by placing the leave in drying oven until the stability of the weight.

Data Analyses

In the case of paired variables, as temporal variations of proline content in both sites for example, Wilcoxon tests were conducted. In the case of several factors possibly involved in the variation of a quantitative variable, the general linear model (G.L.M.) was chosen over several-way ANOVA in order to keep the maximum degree of freedom. All these tests were conducted using SYSTAT 12 (Systat Software Inc. 2007).

The significance of correlations between two variables sharing a normal distribution was tested from the probability associated to Pearson's r coefficient. The calculations of the lag and the associated probability between the temporal variations of two variables were performed using PAST 2.11 (Hammer *et al.*, 2001). Component Analyses were applied to biochemical compounds and metabolic markers in order to visualize the respective positions of all the phenotypes, using PAST 2.11. This software gave also the predictions of values in Zéralda through multiple regression methods established using the data recorded at Médéa.

RESULTS

Description of Poplar Aphid Life Cycle

Chaitophorus leucomelas Koch is an aphid presenting 5 phenotypes, living in colonies on the young branches, the petioles and the leaves of *Populus nigra*. From the beginning of March in the area of Médéa, the eggs of winter hatch and give birth to larvae of fundatrix, which will move towards the petioles and the base of the young leaves. The gradual flow of hatching announces the installation of the first colonies of adult fundatrix. In Zéralda, the appearance of the fundatrix is observed only from the first decade of April (crosscorrelation test, lag=1, p=8.50×10^{-4}) (Table 1).

**Table 1: Temporal Shift in the Phenotype Phenology of Chaitophorus
Leucomelas between the Two Sites**

Phenotypes	Cross Correlation Test		
	Barycenter	Lag	Probability
Fundatrix at Médéa Fundatrix at Zéralda	3.22 4.19	1	8.50×10^{-4}**
Apterouss fundatrigeniae at Médéa Apterouss fundatrigeniae at Zéralda	4.35 5.67	1	5.09×10^{-52}***
Alate fundatrigeniae at Médéa Alate fundatrigeniae at Zéralda	5.56 6.32	1	6.11×10^{-9}***
Adult virginogeniae at Médéa Adult virginogeniae at Zéralda	6.17 6.74	0	0.0121*
Adult sexuparae at Médéa Adult sexuparae at Zéralda	9.43 9.68	0	2.87×10^{-6}***
Sexual adult males at Médéa Sexual adult males at Zéralda	11.53 11.28	0	7.19×10^{-7}***
Sexual adult females at Médéa Sexual adult females at Zéralda	12.00 12.00	0	2.42×10^{-56}***

* :<0,05, ** :<0,01, *** :<0,001

The colonies of fundatrix will produce the generation of fondatrigeniae, i.e. apterous. They will appear around mid-April at Médéa and beyond mid-May at Zéralda (crosscorrelation test, lag=1, p=5.09×10^{-52}) (Table 1). After maturation, their offspring resulting from parthenogenesis colonize the leaf surface and found new colonies. The succession of apterous females will give rise to the alate form around mid-May at Médéa and during the first decade of June at Zéralda (crosscorrelation test, lag=1, p=6.11×10^{-9}) (Table 1). The transitory nymphal stage will lead to adult alate

fondatrigeniae. The fondatrigeniae will make succeed several generations in apterous and winged forms. The latter will establish other colonies at more distant places, thanks to their flight capacity.

The installation of the virginogene phenotype is observed in both areas, with a temporal shift of around ten days between Médéa (at the beginning of June) and Zéralda (mid-June) (crosscorrelation test, lag=0, p=0.0121) (Table 1). Adults are able through several parthenogenetic reproduction episodes to produce several generations throughout the summer period until August. The reproductive sequence of the virginoparae, leads the colonies to be installed on the two faces of the limb, with a direct consequence, the appearance of the alate virginiae. The populational outbreak of the various biological states of virginiae, allows an intense production of honeydew, which causes the myrmecophilous behavior of a particular species of ant *Tapinoma nigerrimum* whose colonies covet regularly the populations of *Chaitophorus leucomelas*.

The phenotype of the sexuparae is generated during the first decade of September at Médéa and in the second decade of the same month at Zéralda (crosscorrelation test, lag=0, p=2.87×10^{-6}) (Table 1). Appearance of the female sexuparae mark a phase of transition from the parthenogenetic mode to the sexual mode. Indeed they represent the last stage of the parthenogenetic reproduction in the cycle of *C. leucomelas*.

The sexual males appear in the two areas roughly at the same time (at the end of November). They represent the beginning of the only sexual phenotype at *C. leucomelas*. From the month of December on in the two sites, the sexual oviparae females become visible. After fecundation, they leave the colonies to find egg laying sites in anfractuosities of the trunks of *Populus nigra*, in bud glumes or in galls abandoned of *Pemphigus imunis*. The laid eggs will spend the winter, and hatch in the beginning of spring.

In summary, the first phenotypes (Fundatrix, Fundatrigeniae of the 1st generation and of the 2nd generation) of *C. leucomelas* appear later in Zéralda than in Médéa. From the Virginogeniae on, this lag is lost until the end of the cycle (Figure 3).
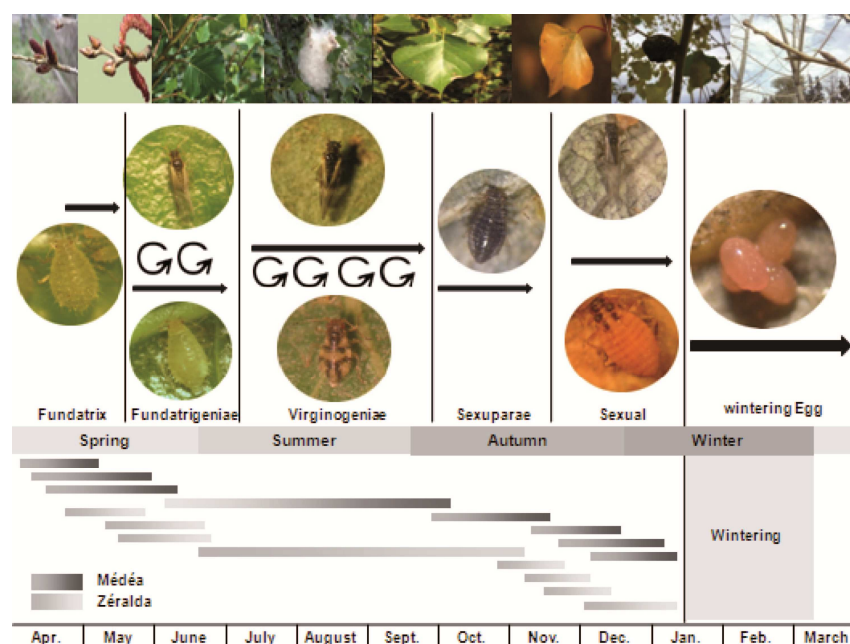

Figure 3: Synthetic Diagram of the Cycle of Life *Chaitophorus leucomelas*

Foliar Phytochemical Products, Rehandling of the Energetic Reserves and Variability of the Fecundity of the Phenotypes of Chaitophorus leucomelas

In order to give a synthetic view of data gathered in table 2, PCAs were conducted for each station.

Table 2: Analyses of Metabolic Markers in Aphids and Biochemical Compounds in Poplar Leaves

Sites	Months	Phenot.	Lipidic Res.	Carboh. Res.	Fecund.	Foliar Carboh.	Cond. Tannins	Proline	Humidity
Médéa	III	FNA	1,452	0,633	0,436	1,928	0,270	0,010	28,740
	IV-V	FDGG1	0,762	0,113	0,148	1,750	0,230	0,017	27,707
	VI-VII	FDGG2	0,537	0,533	0,993	0,903	0,203	0,044	22,927
	VII-IX	VIRA	0,333	0,021	0,063	0,569	0,173	0,140	17,030
	X-XI	SXPA	0,866	0,317	0,366	0,974	0,150	0,040	16,297
	XI-XII	SXA	0,621	0,266	0,429	0,312	0,073	0,018	14,190
Zéralda	III	FNA	0,989	0,325	0,328	1,166	0,314	0,059	26,997
	IV-V	FDGG1	0,864	0,199	0,231	1,070	0,240	0,104	23,967
	VI-VII	FDGG2	0,288	0,077	0,267	0,233	0,193	0,157	20,760
	VII-IX	VIRA	0,486	0,061	0,125	0,498	0,160	0,301	14,900
	X-XI	SXPA	0,549	0,068	0,125	0,350	0,123	0,083	17,480
	XI-XII	SXA	0,278	0,115	0,370	0,223	0,052	0,081	13,690

Phenot: Phenotypes; AFN: Adult Fundatrix, FDGG1: Fondatrigeniae, 1st generation, FDGG2: Fondatrigeniae, 2nd generation; AVIR: Adult virginogeniae, ASXP: Adult sexuparae, SXA: Sexual adults; Lipidic res: Lipidic reserves (µg/mg.F.M); Carboh-Res: Carbohydrate reserves (µg/mg. F.M); Fecun.: Fecundity (larvae numbers/female numbers); Cond. tannins: Condensed tannins (mg/g. F.M.); Foliar carboh.: Foliar carbohydrates (mg/g. F.M.); Proline (µg/g. F.M); Humidity (mg./g. F.M.).

In figure 4A, the projection corresponding to Médéa station shows 4 groups of correlated variables: (i) the foliar carbohydrates, positively linked to humidity and condensed tannins, are associated to the Fundatrigeniae of the 1st generation (FDGG1); (ii) the lipidic reserves stored in aphids, (iii) the carbohydrate reserves of aphids and fecundity show their highest values in the Fundatrigeniae of the 2nd generation (FDGG2); and (iv) the proline, negatively correlated to the group carbohydrate reserve and fecundity, is associated to virginogeniae adults (VIRA). The fundatrix (FNA) are characterized by high values for most variables, except proline measured in leaves. Sexuparae (SXPA), and Sexuals (SXA) on a greater extent, are present when there is a low level of condensed tannins, foliar carbohydrates and humidity.

In figure 4B, only three groups of variables can be described: the 1st group is composed of condensed tannins, foliar carbohydrates, lipidic reserves, carbohydrate reserves and humidity, and associated to Fundatrigeniae of the 1st generation and for their highest values to fundatrix; in contrast, the sexuparae and Fundatrigeniae of the 2nd generation are associated to low values for these 5 variables; the 2nd group corresponds to fecundity, and the 3rd one to Proline. The adult virginogeniae are associated to high values of proline but a low fecundity. The Sexuals develop when the trophic resources are of poor quality.

In summary, the positions of the first (FNA and FDGG1) and last (SXA, SXPA and VIRA) phenotypes do not vary on the projection, whereas the FDGG2 changes. In Médéa, this phenotype was associated to high carbohydrate reserves and fecundity, while in Zéralda, it was found when the trophic resources were low.
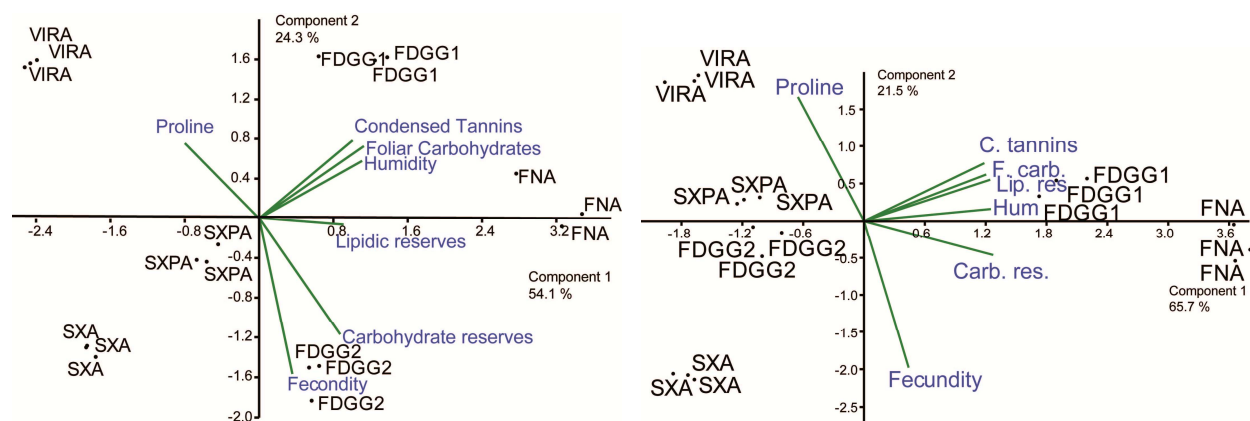
**Figure 4: Principal Component Analyses of Energetic Reserves, Fecundity and
Foliar Biochemical Contents at Médéa (A) and Zéralda (B)**

We further focused on the variables involved in fecundity variation at Médéa. A first multiple regression considering all the foliar and aphid variables (data not shown) highlighted the carbohydrate reserves as the only significant factor (p=0.01). In turn, the carbohydrate reserves are correlated positively to lipidic reserves (p=0.045) negatively to proline (p=0.017). Moreover the lipidic reserves are positively correlated to most variables (the strongest link with foliar carbohydrates with p=0.00024) and negatively to proline (p=0.006).

As a consequence, we compared the values of lipidic and carbohydrates in aphids, fecundity and foliar carbohydrates in both stations through a GLM, taking into account the different phenotypes (Table 3).

Table 3: G.L.M. for Energetic Reserves, Fecundity and Foliar Total Sugars (N=36)

Dependent Variables	Factors	Médéa	Zéralda	F-ratio	P
Lipidic reserves	Station	0.803	0.576	5.53	0.026 *
	Phenotypes			10.80	$<10^{-3}$ ***
Carbohydrates reserves	Station	0.314	0.141	22.01	$<10^{-3}$ ***
	Phenotypes			10.94	$<10^{-3}$ ***
Fecundity	Station	0.406	0.241	10.30	0.003 **
	Phenotypes			9.09	$<10^{-3}$ ***
Foliar carbohydrates	Station	1.073	0.590	74.90	$<10^{-3}$ ***
	Phenotypes			57.94	$<10^{-3}$ ***

These four variables show significant higher values in Médéa than in Zéralda. However, it should be interesting to test if the aphid parameters in Zéralda are "logic" and could be predicted with a certain confidence from the trophic resources of poplar leaves. So we undertook to compare the observed values differed from predicted ones. In the model, the equations presented in table 4 established in from Médéa station were used. First, the lipidic reserves depend on foliar carbohydrate with an associated p-value of $2.4\ 10^{-4}$. Second, the carbohydrate reserves are correlated to lipidic reserves with a p-value of 0.045. Third, fecundity can be expressed as a linear combination of both carbohydrate reserves (positive, $p<10^{-3}$) and lipidic reserves (negative, $p=2.2\ 10^{-3}$) (Table 4).

Table 4: Predicting Equations Established at Medea (N=18)

Lipidic Reserves	Coeff.	Std.err.	p
Constant	0.231	0.138	
Carbohydrate reserves	0.533	0.113	$2.40\ 10^{-4}$ ***
Carbohydrate reserves	Coeff.	Std.err.	p
Constant	0.111	0.105	

Table 4: Contd.,			
Lipidic reserves	0.253	0.116	$4.50 \ 10^{-2}$ *
Fecundity	Coeff	Std.err.	p
Constant	0.29119	0.085647	
Carbohydrate reserves	1.3488	0.19648	$5.37 \ 10^{-6}$ ***
Lipidic reserves	-0.38437	0.10432	$2.21 \ 10^{-3}$ **

Using the standard error associated to each coefficient, we constructed the figure 5. The observed lipidic reserve variations fall in the range of predicted values. In contrast, the observed carbohydrate reserves are lower than the lowest predicted values from the FDGG1 phenotype one. As for fecundity, the observed values are within the range of predicted values for most phenotypes, except for Virginogeniae (VIRA) adults and Sexuparae (SXA) where observed fecundity is more moderate.
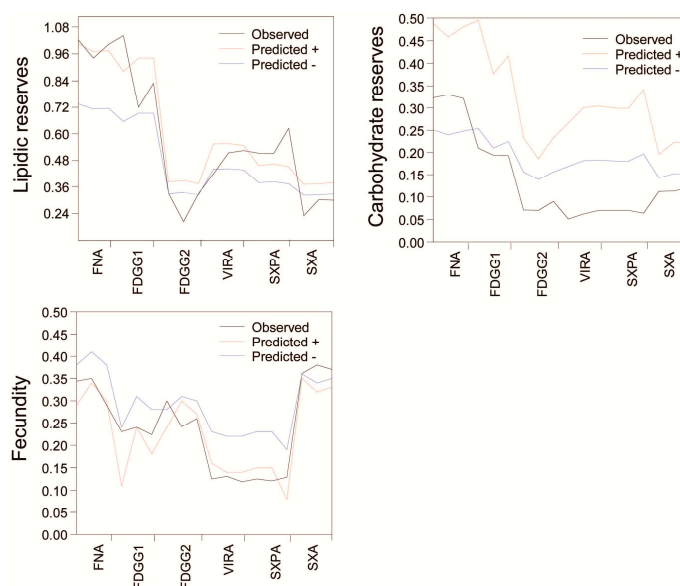

Figure 4: Comparisons between Observed and Predicted Characteristics of Zéralda Aphids

DISCUSSIONS

Seasonal and Geostational Effects on Phenotypical Plasticity of *Chaitophorus Leucomelas*

Chaitophorus leucomelas is a monoecious holocyclic aphid living on black poplar leaves. Its cycle is complex, with numerous phenotypes, and ends in overwintering eggs in Algeria. Our results constitute substantial information concerning the relation between *C. leucomelas* and its environment. The two study stations show differences in their climatic parameters, mainly by the colder winter at Médéa (mountainous area) than at Zéralda (coastal area) and a summer drought spread over a longer time at Zéralda. The poplars settled at Médéa benefit from the advantageous conditions relating to the winter temperatures as the accumulated hours of cold induce the break of bud dormancy (Champagnat, 1983), which is a direct consequence of altitude, earlier than at Zéralda. A shortage of low temperatures produces morphological anomalies (Guyot 1997), and the trees will show a delay in bud breaking (Bottlikova, 1974). As a result, we can predict that aphid life cycle should be more precocious at Médéa in spring. On the other hand, the prolongation of summer drought in Zéralda should lead to a decrease of fitness in the sexual phenotypes.

The study of the different phenotypes enabled us to detect a temporal shift between both stations and it is possible to decipher the potential role of temperatures and rainfalls. The early bud breaking at Médéa directly influences the

appearance of the aphid spring phenotypes, i.e. the fundatrix and the fondatrigeniae, which renew their activity at the end of March at Médéa and in the beginning of May at Zéralda.

This synchronization was already described by Lechowicz (1984) who indicated that the insects which feed on the young leaves of deciduous trees begin their activity in a 3-5 weeks period after the development of the leaves, i.e. when the nutrient quality of foliage is high. A too early hatching is harmful because the larvae will lack food before the emergence of the leaves, but a too late one will be problematic only if there is a short temporal window during which the foliage is of high-quality. In contrast, if the nutrient quality of foliage remains high for a relatively long period, the possibility of larval establishment will be higher, and thus the phenologic correspondence between the insects and their hosts will be flawed (Hunter, 1991).

The autumn-winter generations, i.e. the sexuparae and sexuals appear earlier at Zéralda than at Médéa, showing shorter generations and thus making up the lost time due to late starting. The faster larvae development and succession of short duration generations with increasing temperature are a well known phenomenon (Chararas, 1972; Benassy, 1975).

Relationships between the Phytochemical Quality of the Host Plant Populus Nigra, the Reserve Lipids, and Fecundity of *Chaitophorus leucomelas* Females

In order to clarify the pathways between the different factors influencing ultimately female fecundity and fitness, we present in figures 5a and 5b a tentatively synthesis, from the tables A and B in appendix. In these diagrams, the width of the arrows takes into account the strength of correlations. At a first glance, we observe more relationships within the different elements at Zéralda than at Médéa. We interpret this tendency by more accentuated limiting factors at Zéralda: the least change in the pathway in this station will affect the next step. It remains that the origin of limiting conditions from one hand, and the link between the different steps needs to be explained.

Awmack & Leather (2002) estimate that the food requirements of the insects depend on phytochemical quality of the host plant, which can express foreseeable variations under the effect of the seasonal changes, or unforeseeable ones, such as the changes caused by environmental effort. Moreover, Paquin (1986), reports that the aggressions by the dryness thus have synergic effects on the foliar sugar content. These considerations corroborate the phenologic window of susceptibility advanced by Feeny (1976), Lawrence *et al.* (1997) and Hunter & Elkinton (2000), who stipulate that the biochemical temporal variations in the leaves have a very marked influence on the nutrition and reproductive success of phytophagous insects.

The differences between the total sugar content in the leaves at Médéa and Zéralda are in addition increased by the effect of the adverse conditions. This last point would evoke different strategies installation by the females when they are subjected to stressing conditions. Indeed, the quantities and the quality of the energy allocations between areas seem to be superimposed with the fluctuations of foliar total sugars, in answers to the effect of the dry periods on the foliar total sugar expression.

The difference of fitness between the phenotypes of the two areas of study can be justified by work of Levins (1968) and Convey (1992), which report that the availability of the lipido-carbohydrates reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as the reproduction, maintenance and the growth. The resource invested in the physiological functions thus will enter in conflict with the maintenance of homeostasis. Indeed, according to the principle of allocation, any additional investment in an unspecified aspect of the life

of an organism will not be able to be made without a shortage of energy devoted to another aspect. The phenotypes living with Zéralda show a significant loss in energy reserves because of physiological and behavioral accommodations dictated by high summer temperatures.

The use of the lipidic reserves in the insects was re-examined on several occasions by work of Ziegler & Van Antwerpen (2006); Lalouette *et al.* (2007) and Zhou & Misfields (2009). On the one hand, following a stress, the energy needs are important, and are consequently mobilized to be used as energy source; this phenomenon takes place however with the detriment of their structural or functional role (Futuyma, 2001; Begon *et al*, 2006). Moreover, the individuals exposed to a periodic seasonal desiccation produce a thicker waxy cuticle, including lipids, and its structure is positively correlated with resistance to desiccation. Otherwise, tannins are well-known to lower the growth rate of the herbivorous insects because of their capacities to bind to plant proteins and intestinal.

Through the results obtained, even if has been shown that tannins have negative effects on fecundity, we suggests that these molecules are doubtfully significant, given the low concentrations of condensed tannins in *Populus nigra*, as reported by Zucker (1982). Some studies (Waterman, 1988, quoted by Grayer *et al.*, 1992), show that the formation of insoluble tannin-protein complexes in the intestinal duct of insects are highly unlikely due to the detergent activity of the intestinal fluids and the excessively high levels necessary to discourage insects to feed. Nevertheless, we suspect that the positive correlation observed between condensed tannins and lipidic reserves is not a cause-effect relationship but rather due to a strong positive correlation between foliar total sugar content and condensed tannins.

As for fecundity, the lipidic reserves are key markers relevant of the good biological performance of the mechanisms and fitness of the various phenotypes of *C. leucomelas*. Additional inter-connections (lipidic/carbohydrates reserves, carbohydrates reserves/fecundity, and condensed tannins/lipidic reserves) observed in the phenotypes of Zéralda, relatively to those of Médéa confirm the existence of conflicts between some physiological traits as the fitness and the residual value of the phenotypes in reaction with the thermal stress. Thus, this work points out the adaptation of the aphid to dryness observed in the littoral area, by an allocation of a part of the energy to the maintenance of homeostasis and consequently to the detriment of the fitness expressed by fecundity.

CONCLUSIONS

Our results showed that altitude directly influences the phenomenon of dormancy of the host *Populus nigra* and consequently it leads to temporal ephemeral time lag in the succession of *Chaitophorus leucomelas* phenotypes. The precocity of fondatrix appearance in mountainous zones characterized by a colder winter is compensated by the important reproductive potential of the sexuparae under the effect of a long dry summer. The fecundity variationsof *Chaitophorus leucomelas* phenotyps seems to be influenced by the lipidic/ carbohydrates energy balance depending on the fluctuations of total sugars and of the foliar condensed tannins. Other studies are required to understand the physiological features related to the sensitivity of *Chaitophorus leucomelas* to hight temperature. It would be interesting to analyze the specific life table including the intrinsic rate of natural increase (rm) and the doubling time of a population (Dt) under the effect of extreme temperatures.

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